DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention]The oxidative damage depressant of red corpuscles in which this invention makes astaxanthin or its ester an active principle, It is related with the foodstuffs which have a stabilization effect of the foodstuffs which have the oxidative damage depressant action of the red corpuscles which make an active principle the hardening inhibitor of red corpuscles, the stabilizing agent of red corpuscles, a blood preservative and astaxanthin, or its ester, the foodstuffs which have a hardening prevention operation of red corpuscles, and red corpuscles.

[0002]

[Description of the Prior Art]Blood is continuously put to oxidative stress irrespective of whether the existence is in the living body. Namely, in [in a state of preservation, the peroxylipid in blood goes up by oxidative stress, antioxidation ability causes a fall, and resulting in hemolysis soon is known, and] in the living body on the other hand, When red corpuscles circulate through the inside of a blood vessel, oxidative stress is always received and the film (red corpuscles) is always exposed to the danger of it being hardened or destroyed and hemolyzing. It is thought that hardening and destruction of these films serve as a cause to which the oxygen supply to brain tissue and a peripheral part is reduced, and the function of these organizations is reduced. Preventing hardening and destruction of red corpuscles Arteriosclerosis, hypertension, diabetes mellitus, It is considered that the disease in connection with cancer, hyperlipidemia, rheumatism, gout, cerebral apoplexy, ischemic heart disease, versicular emphysema, a gastric ulcer, pancreatitis, a nephritis, a cataract, an Alzheimer disease, an allergic disease, aging, etc. is useful as prevention or an object for a therapy. It is reported that vitamin E protects [many] red corpuscles from these oxidative stress conventionally, and it is already widely used in the field of health food etc. Although the

astaxanthin which has antioxidative activity still stronger than this vitamin E was found out in recent years, there is no report about the protective action to red corpuscles. Astaxanthin is a long-chain lipophilicity compound and it not only improving membrane fluidity, but being incorporated into the membrane structure of red corpuscles and carrying out protection stabilization of the red corpuscles from oxidant stress is expected. The astaxanthin whose safety are a compound of natural product origin and is high, or its ester, It is reported that there is an effect which prevents or controls antioxidizing, the arteriosclerosis, the ischemic heart disease, or ischemic brain damage of low density lipoprotein (LDL) in a blood serum (JP,10-155459,A). However, there is no report about astaxanthin or its ester carrying out protection stabilization of the red corpuscles.

[0003]

[Problem(s) to be Solved by the Invention]The oxidative damage depressant of red corpuscles in which this invention makes astaxanthin or its ester an active principle, In the hardening inhibitor of red corpuscles, the stabilizing agent of red corpuscles and a blood preservative, and a row. It aims at providing the foodstuffs which have a stabilization effect of the foodstuffs which have the oxidative damage depressant action of the red corpuscles which make astaxanthin or its ester an active principle, the foodstuffs which have a hardening prevention operation of red corpuscles, and red corpuscles.

[0004]

[Means for Solving the Problem]In order that this invention persons may solve an aforementioned problem, as a result of looking for various compounds which have the operation which prevents oxidative damage to red corpuscles, astaxanthin or its ester found out having the operation which prevents oxidative damage to red corpuscles. Ingesta were made to contain these astaxanthin or its ester, and ingesta which have the operation which controls oxidative damage to red corpuscles were found out. This invention is made based on starting knowledge.

[0005]Namely, an invention concerning claim 1 of this invention is an oxidative damage depressant of red corpuscles which make astaxanthin or its ester an active principle, An invention concerning claim 2 is a hardening inhibitor of red corpuscles which make astaxanthin or its ester an active principle, An invention concerning claim 3 is a stabilizing agent of red corpuscles which make astaxanthin or its ester an active principle, An invention concerning claim 4 is a blood preservative which makes astaxanthin or its ester an active principle, Inventions concerning claim 5 are foodstuffs which have the oxidative damage depressant action of red corpuscles which make astaxanthin or its ester an active principle, Inventions concerning claim 6 are foodstuffs which have a hardening prevention operation of red corpuscles which make astaxanthin or its ester an active principle, and inventions concerning claim 7 are foodstuffs which have a stabilization effect of red corpuscles which make

astaxanthin or its ester an active principle.

[0006]"Astaxanthin or its ester" as used in this invention means what is obtained by a thing of natural product origin, or composition. As a thing of natural product origin, for example A shell and eggs of crustacean, such as a shrimp, a krill, and a crab, What is obtained from Spermatophytes, such as yeast, such as algae, such as an organ, a hide of various fish and shellfishes, eggs, and a hematococcus, and red yeast Phaffia, oceanic bacteria (Agrobacterium aurantiacum) or Fukuju grass, and a buttercup, can be mentioned. An extract from nature and a chemical composition are marketed, and acquisition is easy.

[0007]Astaxanthin or its ester is obtained by cultivating red yeast Phaffia, a green algae hematococcus, oceanic bacteria, etc. by a proper culture medium in accordance with a conventional method based on a publicly known method, for example.

[0008]Various methods are known about a method of extracting astaxanthin from the above-mentioned culture, or a method of extracting and refining from said crustacean. For example, since diester type astaxanthin is an oil soluble substance, oil-soluble organic solvents, such as acetone, alcohol, ethyl acetate, benzene, and chloroform, can extract an astaxanthin component from a natural product containing astaxanthin. After extraction, a solvent can be removed in accordance with a conventional method, and an astaxanthin concentrate of a diester type can be obtained. A request may refine an obtained concentrate further.

[0009]Astaxanthin has the 3,3'-dihydroxy- beta, beta-carotene 4,4'-dione, or its stereoisomeric form. Although three sorts of stereoisomeric forms, (3R,3'R)-astaxanthin, (3R,3'S)-astaxanthin, and (3S,3'S)-astaxanthin, are known, specifically, the all can be used for this invention.

[0010]It is known that astaxanthin or its ester is a compound with high safety in which mutagen nature is not observed.

[0011]An oxidative damage depressant of red corpuscles which make an active principle astaxanthin of this invention, or its ester, Or both educt of astaxanthin mono- and diester can be used for ingesta which have the oxidative damage depressant action of red corpuscles which make astaxanthin or its ester an active principle. Since two hydroxyl groups are protected by ester bond, physically, stability is better than educt and monoester and the oxidative degradation of the diester is hard to be carried out in pharmaceutical preparation. However, if incorporated into a living body, it is promptly hydrolyzed into astaxanthin by enzyme in the living body, and it is thought that effect is taken.

[0012]Ester species with saturated fatty acid low-grade as monoester of astaxanthin or high-class or low-grade or high-class unsaturated fatty acid can be mentioned. Specifically Acetic acid, lauric acid, myristic acid, pentadecanoic acid, pulmitic acid, Palmitoleic acid, heptadecanoic acid, elaidic acid, recinoleic acid, A petroselinic acid, vaccenic acid, eleostearic acid, PUNISHIN acid, licanic acid, Parinaric acid, GADORU acid, 5-eicosenic acid, 5-dococenoic acid, SETORU acid, Monoester, such as erucic acid, 5,13-docosadienoic acid,

cera cholic acid, decenoic acid, stay ring acid, dodecenoic acid, oleic acid, stearic acid, eicosapentaenoic acid, docosahexaenoic acid, linolic acid, linolenic acid, and arachidonic acid, can be mentioned. The diester which comprises fatty acid same or of a different kind chosen from a group of the above-mentioned fatty acid as diester of astaxanthin can be mentioned.
[0013]As ester of astaxanthin, For example, amino acid ester, such as a glycine and an alanine, acetate ester, Monovalence or polyvalent-carboxylic-acid ester, and its salts, such as citrate, Or inorganic acid ester species and its salts, such as phosphoric ester and sulfate ester, Monoester, such as sugar ester, such as glucoside, sugar fatty acid ester, glycero sugar fatty acid ester, SUFINGO sugar fatty acid ester, glycero fatty acid ester, and glycerophosphate, can be mentioned. Or the above-mentioned amino acid, carboxylic acid, phosphoric acid, sulfuric acid, sugar, unsaturated fatty acid, Diester same or of a different kind etc. which are chosen from saturated fatty acid, a higher unsaturated fatty acid, fatty acid ester, sugar fatty acid ester, glycero sugar fatty acid ester, SUFINGO sugar fatty acid ester, glycero fatty acid ester, and glycerophosphate can be mentioned.
[0014]The glycerophosphate which contain the fatty acid chosen from the saturated fatty acid ester or a higher unsaturated fatty acid, unsaturated fatty acid, or saturated fatty acid of glycerophosphoric acid as diester of glycerophosphoric acid can be mentioned.
[0015]As an example of the JIGURI cello phosphoric ester of astaxanthin, astaxanthin JIGURI cello phosphoric ester, Astaxanthin glycerophosphoric acid pulmitic acid, astaxanthin glycero phosphatidylcholine pulmitic acid, The astaxanthin glycero phosphatidylcholine DHA, astaxanthin glycero phosphatidylinositol pulmitic acid, Compounds, such as the astaxanthin glycero phosphatidylinositol DHA, astaxanthin glycero phosphatidylinositol linolic acid, and astaxanthin glycero phosphatidylcholine linolic acid, can be mentioned.
[0016]
[Example]Although the following reference examples and examples explain this invention in detail, this invention is not limited to these.
[0017]Example 1 The depressant action (i) test-method astaxanthin of the astaxanthin to rat erythrocyte membrane oxidative damage or its ester is added to rat red blood cell suspension, and it incubates. If 2,2'-azobis (2-amidinopropane) dihydrochloride (following, AAPH) which is a free radical generating agent is added, hemolysis will occur with time progress. It was investigated whether astaxanthin or its ester would suppress hemolysis.
(ii) Put 6 mg of preparation astaxanthin (made by a sigma company) of astaxanthin liquid and control liquid into a mortar, grind finely, and it is HCO-60. It could be 100 ml using 2 g and a physiological salt solution. It is HCO-60 as control liquid. It could be 10 ml using 0.2 g and a physiological salt solution.
(iii) The preparation Wistar system male rat of erythrocyte suspension (RBC) It used that preliminary breeding for about one month after carrying in was performed, and there were no

abnormalities in general status in [3 ** Lab Service], having checked. It accommodated five animals at a time in the cage made from a wire gauze, and bred in the temperature of 22**2 **, 55**10% of humidity, and the rearing room set up in lighting 12 hours (7:00 a.m. to 7:00 p.m.). A pellet and water were made to take in freely. The weights at the time of an experiment were 314-385g. It collects blood from an abdominal aorta under anesthesia, and 2%EDTA-2K of 1/10 quantity is mixed. Plasma and a buffy coat were removed after centrifugal separation for 10 minutes at 1000 g. This operation that adds a phosphoric acid buffer physiological salt solution (PBS:125mM sodium chloride, 10mM sodium phosphate, pH 7.4) of the 5 time capacity of the red corpuscles which remain, mixes, centrifuges for 10 minutes at 1000 g, and removes supernatant liquid was repeated 3 times, and red corpuscles were washed. PBS of the 4 time capacity of red corpuscles was added to this, and erythrocyte suspension (RBC) was prepared 20%.

[0018](iv) The measurement experiment of the grade of hemolysis was divided into four groups, and was conducted. That is, they could be nine examples each by astaxanthin 0.1muM, 0.5microM, 1.0microM, and a control group (0microM). As shown in the following table 1, each liquid (ml) was put into the test tube at each group.

[Table 1]

	コントロール群	0.1μM群	0.5μM群	1.0μM群
アスタキサンチン液	0.0	0.01	0.05	0.10
コントロール液	0.20	0.09	0.15	0.10
RBC	5.0	5.0	5.0	5.0
PBS	4.8	4.8	4.8	4.8
総量	10.0	10.0	10.0	10.0

[0019]It incubated shaking these test tubes for 37 ** and 30 minutes in a dark place. Next, it centrifuged for 10 minutes at 1000 g, and supernatant liquid was removed, 10-ml PBS was added, reversal mixture was carried out, same centrifugal separation was performed after that, ****** was taken and removed, and red corpuscles were washed. This washing operation was repeated 3 times. After-washing PBS was added, it was referred to as 5 ml and 5 ml of 150mM AAPH PBS solutions were added, and it incubated, shaking for 37 ** and 30 minutes in a dark place. After incubating for 90 minutes, it extracted in every 0.1-ml two test tubes, and in order to hemolyze thoroughly 4-ml PBS (solution A) and another side to one side, 4 ml of distilled water was added (solution B). Both were centrifuged for 5 minutes at 2000 g, and the absorbance at 540 nm of supernatant liquid was measured. The grade of hemolysis was computed by the following formula.

Grade of hemolysis (%) =(absorbance of absorbance / solution B of solution A) x100 [0020] The effect of astaxanthin over rat erythrocyte membrane oxidative damage is shown in Table

2.

[0021]

[Table 2]

アスタキサンチン (μM)	例数	溶血度 (%)
0 (対照群)	9	79.18 ± 7.29
0.1	9	75.56 ± 9.46
0.5	9	66.81 ± 8.57**
1.0	9	61.01 ±10.74**

Each value is average value ** standard deviation **:p<0.01. Administration group vs. Control group (t-test)

[0022]the hemolysis caused when astaxanthin adds AAPH to rat red corpuscles from the result of the above-mentioned table 2 -- a dosage -- it turns out that anaclitic and it controls with statistical significance.

[0023]It turned out that astaxanthin shows depressant action to erythrocyte membrane oxidative damage by this invention, and red corpuscles are stabilized. That is, astaxanthin or its ester is understood that it is useful as an oxidative damage depressant of the red corpuscles made into an active principle.

[0024]The oxidative damage depressant of the red corpuscles of this invention Stabilization of the red corpuscles at the time of blood preservation, And the disease to which the oxidative damage to red corpuscles is said as the cause, for example, arteriosclerosis, It is useful as prevention of hypertension, diabetes mellitus, cancer, hyperlipidemia, rheumatism, gout, cerebral apoplexy, ischemic heart disease, versicular emphysema, a gastric ulcer, pancreatitis, a nephritis, a cataract, an Alzheimer disease, an allergic disease, aging, etc., or a treating agent. It is useful also as a blood preservative.

[0025]In accordance with a conventional method, the drugs of this invention Sugar, such as proper lactose and saccharose, Excipients, such as amino acid, such as a glycine, and cellulose, starch, gelatin, Lubricant, such as disintegrator, such as binding materials, such as methyl cellulose and a polyvinyl pyrrolidone, or starch, and agar, or a silicon dioxide, talc, magnesium stearate, and a polyethylene glycol, a flavor agent, and sweetners can be blended, and it can be made the gestalt of various pharmaceutical preparation. For example, a medicine is prescribed for the patient with a fluid medication gestalt like a solid medication gestalt like powder, such as a tablet, granulation, and a fine grain, an elixir, syrup, and suspension. The extract or crude extract of natural product origin may be sufficient also as a chemical composition, the astaxanthin or ester used as an active principle is independent, or it can mix suitably and it can use these.

[0026]Astaxanthin or its ester tends to receive the oxidative degradation by oxygen in the air, and when physical stability, such as temperature and light, is bad and considers it as pharmaceutical preparation, it will be temporally decomposed during a retention period and it

will be deactivated. Since decomposition of this astaxanthin or its ester is suppressed, if necessary, the substance which has antioxidation ability in the above-mentioned constituent as stabilizer can be added. For example, vitamin A, vitamin B, vitamin C, vitamin E, or such vitamers, A kind or two sorts or more of mixtures chosen from the existing anti-oxidants, such as cystein, glutathione, glutathione peroxidase, citrate, phosphoric acid, polyphenol, nucleic acid, Chinese orthodox medicine, seaweed, and an inorganic substance, can also be added. In order to improve absorption of the simple substance of astaxanthin, or a monoester object, it is preferred to use fine-powder-form voice and to prescribe a medicine for the patient.

[0027]The quantity of the astaxanthin used as drugs or its ester is an astaxanthin equivalent unit, and the dose of 1 mg - 20 mg performs it preferably 0.5 mg - 100 mg per day by adult at internal use or parenteral administration. A dose changes with a patient's age prescribed for the patient, weight, the grade of condition, and dosage forms.

[0028]The ingesta which have the oxidative damage depressant action of the red corpuscles which make astaxanthin or its ester an active principle are also contained in this invention.

[0029]As an example of addition to ingesta, margarine, butter, butter sauce, a cheese head, Whipped cream, shortening, lard, ice cream, yogurt, Dairy products, sauce processed meat, fish products, a fried potato, potato chips, Popcorn, fish flour, chewing gum, chocolate, a pudding, Jelly, a fruit gum, a candy, drops, a caramel, Sponge cake, a cake, a doughnut, a biscuit, Cookie, a cracker, etc., Macaroni, paste, vegetable oil, instant soup, a dressing, The example of addition to common foodstuffs, such as alcoholic beverages, such as non-alcohol, such as tea, such as carbonic acid system drinks, such as fruit-juice drinks, such as an egg, mayonnaise, and bean paste, a soft drink, and a sport drink, or a non-carbonic acid system drink, coffee, and cocoa, or liqueur, and medicinal drinks, can be given.

[0030]The ingesta of this invention can blend astaxanthin or its ester with the raw material of common foodstuffs, and processing manufacture can be carried out in accordance with a conventional method. Although the loadings of astaxanthin or its ester change with gestalten of foodstuffs, etc., generally, 0.001 to 10%, it is 0.01 to 5% preferably, and it prepares so that only a complement may be contained in demonstrating erythrocyte membrane oxidative damage depressant action. A person skilled in the art can choose the amount used suitably according to the kind of ingesta.

[0031]When using the ingesta of this invention as a supplement or functional food, the gestalt may be the same gestalt as the above-mentioned pharmaceutical preparation for medicines. The egg white oligopeptide which are these decomposition products, such as milk protein, soybean protein, and ovalbumin protein, soybean hydrolyzate, and the mixture of an amino acid simple substance can also be used. Works which blended sugars, a fat, a trace element, vitamins, an emulsifier, perfume, etc., such as natural liquid food, defined formula diet and nourishing food, drinkable preparations, a capsule, and an enteral hyperalimentation drug, can

be mentioned. When it provides with a drink gestalt, in order to improve nutritional balance and the flavor at the time of ingestion, nutritional additives, such as amino acid, vitamins, and minerals, sweetners, spices, perfume, coloring matter, etc. may be blended. The gestalt of the foodstuffs of this invention is not limited to these.

[0032]

[Effect of the Invention]The oxidative damage depressant of the red corpuscles which make astaxanthin or its ester an active principle by this invention, The foodstuffs which have a stabilization effect of the foodstuffs which have the oxidative damage depressant action of the red corpuscles which make an active principle the hardening inhibitor of red corpuscles, the stabilizing agent of red corpuscles, a blood preservative and astaxanthin, or its ester, the foodstuffs which have a hardening prevention operation of red corpuscles, and red corpuscles were able to be provided. The erythrocyte membrane oxidative damage depressant of this invention can prevent hardening and destruction of red corpuscles, In bank blood, improve the stability, and in in the living body Arteriosclerosis, It is useful as a raw material of drugs or functional food as prevention or the object for a therapy of the disease in connection with hypertension, diabetes mellitus, cancer, hyperlipidemia, rheumatism, gout, cerebral apoplexy, ischemic heart disease, versicular emphysema, a gastric ulcer, pancreatitis, a nephritis, a cataract, an Alzheimer disease, an allergic disease, aging, etc.

[Translation done.]